

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph on page 6, line 39 with the following rewritten paragraph:

Figure 4. Intentionally Omitted Expression of 254P1D6b in 293T cells. Figure 4A. 293T cells were transfected with either an empty pCDNA 3.1 vector plasmid or pCDNA 3.1 plasmid encoding the full length cDNA of 254P1D6b. 2 days post- transfection, lysates were prepared from the transfected cells and separated by SDS-PAGE, transferred to nitrocellulose and subjected to Western blotting using an anti-His pAb (Santa Cruz Biotechnology, Santa Cruz, California) to detect the C-terminal epitope tag on the protein. An arrow indicates the band corresponding to the full length 254P1D6b protein product. An additional verified lysate containing an epitope tagged AGSX protein served as a positive control. Figure 4B. 293T cells were transfected with either an empty vector or the Tag5 expression vector encoding the extracellular domain (ECD) of 254P1D6 (amino acids 26-953) and subjected to SDS-PAGE and Western blotting as described above. An arrow indicates the band corresponding to the 254P1D6b ECD present in the lysates and the media from transfected cells.

Please delete the paragraph on page 8, lines 20-28:

~~**Figure 17. Expression of 254P1D6b in 293T cells. Figure 17A.** 293T cells were transfected with either an empty pCDNA 3.1 vector plasmid or pCDNA 3.1 plasmid encoding the full length cDNA of 254P1D6b. 2 days post transfection, lysates were prepared from the transfected cells and separated by SDS PAGE, transferred to nitrocellulose and subjected to Western blotting using an anti His pAb (Santa Cruz Biotechnology, Santa Cruz, California) to detect the C terminal epitope tag on the protein. An arrow indicates the band corresponding to the full length 254P1D6b protein product. An additional verified lysate containing an epitope tagged AGSX protein served as a positive control. **Figure 17B.** 293T cells were transfected with either an empty vector or the Tag5 expression vector encoding the extracellular domain (ECD) of 254P1D6 (amino acids 26-953) and subjected to SDS PAGE and Western blotting as described above. An arrow indicates the band corresponding to the 254P1D6b ECD present in the lysates and the media from transfected cells.~~

Please replace the paragraph on page 87, lines 3-8, with the following rewritten paragraph:

The complete ORF of 254P1D6B v.2 was cloned into the pcDNA3.1/MycHis construct to generate 254P1D6B.pcDNA3.1/MycHis. ~~Figure 4A~~ ~~Figure 17A~~ shows expression of 254P1D6B.pcDNA3.1/MycHis following transfection into 293T cells. 293T cells were transfected with either 254P1D6B.pcDNA3.1/MycHis or pcDNA3.1/MycHis vector control. Forty hours later, cell lysates were collected. Samples were run on an SDS-PAGE acrylamide gel, blotted and stained with anti-his antibody. The blot was developed using the ECL chemiluminescence kit and visualized by autoradiography. Results show expression of 254P1D6B from the 254P1D6B.pcDNA3.1/MycHis construct in the lysates of transfected cells.

Please replace the paragraph on page 87, lines 35-40, with the following rewritten paragraph:

The extracellular domain, amino acids 26-953, of 254P1D6B v.1 was cloned into the pTag5 construct to generate 254P1D6B.pTag5. ~~Figure 4B~~ ~~Figure 17B~~ shows expression and secretion of the extracellular domain of 254P1D6B following 254P1D6B.pTag5 vector transfection into 293T cells. 293T cells were transfected with 254P1D6B.pTag5 construct. Forty hours later, supernatant as well as cell lysates were collected. Samples were run on an SDS-PAGE acrylamide gel, blotted and stained with anti-his antibody. The blot was developed using the ECL chemiluminescence kit and visualized by autoradiography. Results show expression and secretion of 254P1D6B from the 254P1D6B.pTag5 transfected cells.

Please replace the paragraph spanning pages 90 to 91 with the following rewritten paragraph:

In addition to bacterial derived fusion proteins, mammalian expressed protein antigens are also used. These antigens are expressed from mammalian expression vectors such as the Tag5 and Fc-fusion vectors (see the section entitled "Production of Recombinant 254P1D6B in Eukaryotic Systems"), and retains post-translational modifications such as glycosylations found in native protein. In one embodiment, amino acids 26-953 of 254P1D6B variant 1 was cloned into the Tag5 mammalian secretion vector, and expressed in 293T cells (~~Figure 4~~ ~~Figure 17~~). The recombinant protein is purified by metal chelate chromatography from tissue culture supernatants of 293T cells

stably expressing the recombinant vector. The purified Tag5 254P1D6B protein is then used as immunogen.

Please replace the paragraph on page 91, lines 12-20, with the following rewritten paragraph:

To test reactivity and specificity of immune serum, such as the rabbit serum derived from immunization with the GST-fusion of 254P1D6B variant 1 protein, the full-length 254P1D6B variant 1 cDNA is cloned into pCDNA 3.1 myc-his expression vector (Invitrogen, see the Example entitled “Production of Recombinant 254P1D6B in Eukaryotic Systems”). After transfection of the constructs into 293T cells, cell lysates are probed with the anti-254P1D6B serum and with anti-His antibody (Santa Cruz Biotechnologies, Santa Cruz, CA) to determine specific reactivity to denatured 254P1D6B protein using the Western blot technique (Figure 4~~Figure 17~~). In addition, the immune serum is tested by fluorescence microscopy, flow cytometry and immunoprecipitation against 293T and other recombinant 254P1D6B-expressing cells to determine specific recognition of native protein. Western blot, immunoprecipitation, fluorescent microscopy, and flow cytometric techniques using cells that endogenously express 254P1D6B are also carried out to test reactivity and specificity.

Please replace the paragraph on page 92, lines 24-37, with the following rewritten paragraph:

In one embodiment for generating 254P1D6B monoclonal antibodies, a GST-fusion of variant 1 antigen encoding amino acids 21-182 is expressed and purified from bacteria. Balb C mice are initially immunized intraperitoneally with 25 µg of the GST-254P1D6B variant 1 protein mixed in complete Freund’s adjuvant. Mice are subsequently immunized every two weeks with 25 µg of the antigen mixed in incomplete Freund’s adjuvant for a total of three immunizations. ELISA using the GST-fusion antigen and a cleavage product from which the GST portion is removed determines the titer of serum from immunized mice. Reactivity and specificity of serum to full length 254P1D6B variant 1 protein is monitored by Western blotting, immunoprecipitation and flow cytometry using 293T cells transfected with an expression vector encoding the 254P1D6B variant 1 cDNA (see e.g., the Example entitled “Production of Recombinant 254P1D6B in Eukaryotic Systems” and Figure 4~~Figure 17~~). Other recombinant 254P1D6B variant 1-expressing cells or cells

endogenously expressing 254P1D6B variant 1 are also used. Mice showing the strongest reactivity are rested and given a final injection of antigen in PBS and then sacrificed four days later. The spleens of the sacrificed mice are harvested and fused to SPO/2 myeloma cells using standard procedures (Harlow and Lane, 1988). Supernatants from HAT selected growth wells are screened by ELISA, Western blot, immunoprecipitation, fluorescent microscopy, and flow cytometry to identify 254P1D6B specific antibody-producing clones.